Variant ALDH2 is associated with accelerated progression of bone marrow failure in Japanese Fanconi anemia patients

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Key points

- We found the defective ALDH2 variant is associated with accelerated progression of bone marrow failure in Japanese Fanconi anemia patients.
- The data support the view that aldehydes are an important source of genotoxicity in the human hematopoietic system.

Abstract

Fanconi anemia (FA) is a severe hereditary disorder with defective DNA damage response and repair. It is characterized by phenotypes including progressive bone marrow failure (BMF), developmental abnormalities, and increased occurrence of leukemia and cancer. Recent studies in mice have suggested that the FA proteins might counteract aldehyde-induced genotoxicity in hematopoietic stem cells. Nearly half of the Japanese population carries a dominant negative allele (rs671) of the aldehyde-catalyzing enzyme *ALDH2* (acetaldehyde dehydrogenase 2), providing an opportunity to test this hypothesis in humans. We examined 64 Japanese FA patients, and found that the *ALDH2* variant is associated with accelerated progression of BMF, while birth weight or the number of physical abnormalities was not affected. Moreover, malformations at some specific anatomic locations were observed more frequently in *ALDH2*-deficient patients. Our current data indicate that the level of ALDH2 activity impacts pathogenesis in FA, suggesting the possibility of a novel therapeutic approach.

Introduction

Fanconi anemia (FA) is a genomic instability disorder with phenotypes including progressive bone marrow failure (BMF), developmental abnormalities, and increased occurrence of leukemia and cancer[1]. To date 16 genes have been implicated in FA, and their products form a common DNA repair network ("FA pathway")[2,3]. Since FA cells are hypersensitive to DNA interstrand cross-links (ICLs), the FA pathway has been considered to be involved in the repair of ICLs[2,3]. However, it remains unclear what type of endogenous DNA damage is repaired through the FA pathway. Recent studies have suggested that FA cells are also sensitive to aldehydes[4], which may create DNA adducts including ICLs or DNA-protein crosslinks. Furthermore, double knockout mice deficient in *Fancd2* and *Aldh2*, but neither of the single mutant mice, display an accelerated development of leukemia and BMF[5,6]. On the other hand, Fanc-deficient mice in general do not fully recapitulate the human FA phenotype, including overt BMF[7]. Thus, the role of aldehydes in the pathogenesis of human FA is still uncertain.

ALDH2 deficiency resulting from a Glu504Lys substitution (rs671, hereinafter referred to as the A allele) is highly prevalent in East Asian populations. The A allele (Lys504) acts as a dominant negative, since the variant form can suppress the activity of the Glu504 form (G allele) in GA heterozygotes by the formation of heterotetramers[8]. Individuals with the A variant experience flushing when drinking alcohol, and have an elevated risk of esophageal cancer with habitual drinking[9]. Because the frequency of the A allele is close to 50% in the Japanese population at large, some Japanese FA patients are expected to be deficient in *ALDH2*. We thus set out to determine the *ALDH2* status in a collection of Japanese FA patients.

Methods

The onset of BMF was defined according to the criteria used in the International Fanconi Anemia Registry (IFAR) study[10]. Criteria for diagnosis of aplastic anemia and other conditions are described in Supplemental Methods. We observed physical abnormalities characteristic of FA, including skin abnormalities (hyperpigmentation and *café au lait* spots), low birth weight, growth defects, and malformations affecting skeletal systems and deep organs. Extensive malformation was defined as the involvement of at least three sites including at least one deep organ[11]. Mutation analysis of *FANCA/FANCC/FANCG* genes[12], *ALDH2* genotyping[13], Multiplex Ligation-mediated Probe Amplification (MLPA) test for *FANCA* (Falco), and whole exome sequencing (WES)[14] were done as previously described. Details are provided as supplemental methods. Development of BMF or acute myeloid leukemia (AML)/myelodysplasia (MDS) was analyzed by the Kaplan-Meier method or the cumulative incidence method[15,16], respectively, since competing events (e.g., death and stem cell transplantation (SCT)) existed in AML/MDS but not in BMF. This study was approved by the Research Ethics Committee of the Tokai University Hospital and Kyoto University. We obtained family informed consent from all subjects involved in this work.

Results and Discussion

All of the patients in this study (n=64, supplemental Table 1) were referred to the Tokai University Hospital because of pancytopenia, in some cases with MDS or leukemia. The clinical diagnosis of FA was made based on clinical presentation and Diepoxybutane (DEB)-induced chromosome fragility tests in peripheral blood lymphocytes[17], except for three cases in which the DEB test was negative due to *FANCA* reversion mosaicism (supplemental Table 1 and 2). Most of the patients underwent allogeneic SCT, indicating that our patients probably represent a FA population with relatively severe hematological symptoms.

To determine which FA gene was mutated in each of these patients, we applied combinations of PCR-based methods (n=26), the MLPA test for *FANCA* mutations (n=44),

and WES (n=29). In our WES analysis, more than 90% of the 50 Mb target sequences were analyzed by greater than ten independent reads (data not shown). 59 patients were found to have a mutation in FA genes in at least one allele, but 5 of them were mutation-free in the known 16 FA genes, even after WES (Table 1 and supplemental Table 1). These unclassified cases might be caused by large deletions or intronic mutations that are difficult to detect with these methods[18], or possibly mutations in a novel FA gene.

We determined the *ALDH2* genotype in our series of 64 patients (Table 1 and supplemental Table 1). The distribution of the *ALDH2* variant alleles appeared not significantly different from the reported allele frequencies in the healthy Japanese population[13]. The occurrence of leukemia and/or MDS was also not significantly different between patients with GA and GG genotypes. Strikingly, however, we found that progression of BMF was accelerated in heterozygous carriers of the variant A allele compared to homozygous GG patients (Figure 1A and B). Moreover, the three individuals carrying AA alleles developed MDS with BMF at a very young age (Figure 1A and B). None of these three patients belonged to FA-D1 or FA-N, the FA subgroups with severe symptoms[19,20]. Patient number 3 had biallelic frameshift mutations (S115AfsX11) in *FANCP/SLX4*. By contrast, of the FA-P patients that have previously been reported, none have displayed particularly severe symptoms[21-23].

FA is a heterogeneous disorder, and our cohort of patients is quite heterogeneous in terms of complementation groups and types of mutations (Table 1). In order to reduce some of the variability, we selected only the *FANCA* patients having nonsense, frameshift, or large deletion mutations identified at both alleles, (n=12, supplemental Table 1), and repeated the analysis. A patient with probable *FANCA* reversion (patient number 55) was excluded. In this subset of patients, a highly significant statistical difference was reproduced in BMF progression (Figure 1C) but not in AML/MDS development (data not shown).

We could not detect any significant difference in terms of % birth weight (Figure 1D) or number of physical abnormalities (Figure 1E) that correlated with the *ALDH2* genotypes. However, a significant difference was observed in the incidence of each class of malformations in the case of radial, cardiovascular, skeletal, or kidney anomalies, and in the incidence of extensive malformation (Figure 1F).

In conclusion, our current data indicate that endogenous aldehydes are an important source of genotoxicity in the human hematopoietic system, and the FA pathway counteracts them. If the FA pathway is compromised, hematopoietic stem cells (HSCs) likely accumulate aldehyde-induced DNA damage, resulting in BMF due to p53/p21-mediated cell death or senescence[6,24]. Consistent with this model, a recent study showed that the HSCs in *aldh2/fancd2* double knockout mice accumulate more DNA damage than HSCs in either of the single knockout mice[6]. Since some *ALDH2*-proficient FA patients developed BMF early, other modifier genes or environmental factors might affect levels of aldehydes or other genotoxic substances. Interestingly, our data predict that Japanese FA patients in general develop BMF at an earlier age compared to patients of other ethnic origins. We need to establish a Japanese FA registry similar to IFAR to test whether this is true or not. Finally, it seems worth considering *ALDH2* agonists such as Alda-1 as protective drugs against BMF in FA patients. Alda-1 can stimulate the enzymatic activity of both the normal and variant ALDH2[25], suggesting that Alda-1 or a similar drug could be beneficial even for *ALDH2*-proficient FA cases.

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Contributions: M.Y. and H.Y. examined DEB-induced chromosome aberrations, carried out MLPA testing, and analyzed clinical records. K.Y., Y.O., Y.S., K.C., H.K, S.M., S.K., and S.O. performed whole exome sequencing and analyzed sequence data. A.H. validated exome data, and carried out genotyping, and A.H, M.Y., H.Y., K.M., J.N., and M.T. analyzed data. M.Y., M.T., and K.M. wrote the paper.

Conflicts of Interest disclosure: The authors declare that they have no competing financial interests.

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Table 1. Summary of genotypes and clinical characteristics of the patients studied.

		ALDH2 genotype		
	Total	GG	GA	AA
Number of cases	64	36	25	3
Mutated FA gene[A]				
FANCA	39	26[B]	11	2
FANCG	15	7	8	-
FANCI	2	-	2	-
FANCM	1	-	1	-
FANCP	2	-	1	1
Unknown	5	3	2	-
Disease				
AA	2	2	-	-
SAA	40	21	19	-
MDS/AML	22	13	6	3 [C]
Tongue cancer	2	1	1	-
Median months of onset (range)				
BMF	52 (0-297)	72 (27-297)	28 (7-87)	0 (0-7)
MDS/AML	118 (4-384)	156 (61-384)	85 (41-192)	4 (4-12)
Number of cases with SCT (%)	58(91)	33(92)	23(92)	2(67)
Median months at SCT (range)	118 (12-448)	130 (52-448)	86 (28-248)	25 (13-36)

MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; AA, aplastic anemia; SAA, severe aplastic anemia; SCT, stem cell transplantation.

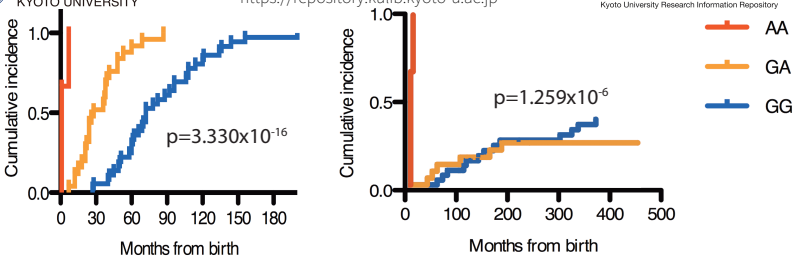
[A] Mutations found in the patients were listed in supplemental Table 1. Some of them were presumptive, since their functional significance has not been determined.

[B] Somatic mosaicism due to reversion was confirmed in two cases and suspected in one case.

[C] In these cases, onset of SAA and MDS was essentially simultaneous.

Figure 1. **Effects of the ALDH2 deficiency on Japanese FA patients. (A** and **B)** Cumulative incidence of BMF (**A**) or MDS/AML (**B**) were analyzed in 64 FA subjects. Numbers of AA, GA, and GG patients were 3, 25, and 36, respectively. (**C**) Cumulative incidence of BMF was analyzed in patients with confirmed biallelic *FANCA* mutations having protein truncations and/or large deletions (n=12). Numbers of AA, GA, and GG patients were 1, 5, and 6, respectively. P-values shown were calculated by Gray's test. In (A), p-values between genotypes were 8.625×10^{-7} (GG vs. GA), 2.107×10^{-10} (GG vs. AA), 1.259×10^{-6} (GA vs. AA), respectively. In (**B**), the difference between GG and GA subjects was not significant (p=0.4564479), whereas other statistical comparisons were highly significant (GG vs. AA, 2.911×10^{-10}; GA vs. AA, 8.813×10^{-8}). In (**C**), the p-values between GG and GA, GG and AA, or GA and AA were calculated as 0.001228433, 0.01430588, 0.02534732, respectively. (**D**) % birth weight or (**E**) total number of physical abnormalities (shown in Supplemental Table 1) in 64 FA patients with three *ALDH2* genotypes. Birth weight was normalized to mean weight at gestational age in Japan. Mean and SEM are indicated. Birth weight records were missing for three patients (supplemental Table 1). There was no significant difference between the *ALDH2* genotypes (Kruskal-Wallis test). (**F**) Frequency (%) of cardiovascular, radial, thumb, skeletal, kidney, and extensive malformations in each *ALDH2* genotype. P-values were calculated by the Cochran-Armitage test for trend, which detects statistical significance of effects across the genotypes. The error bars represent 95% confidence intervals.

B



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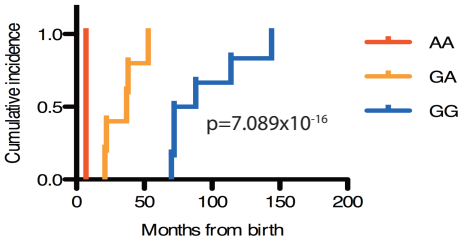

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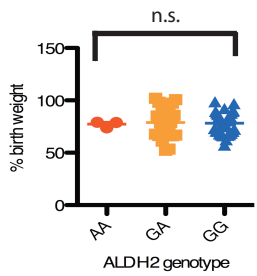

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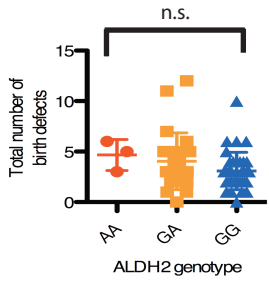

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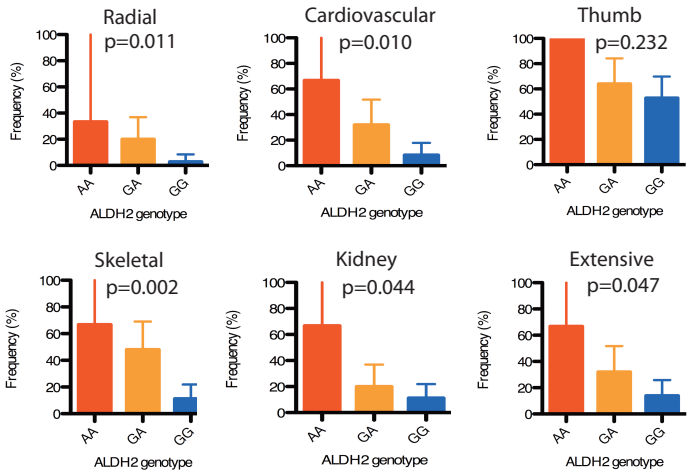

Supplemental Methods

Patients. Diagnosis of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) is based on morphological criteria described by the FAB committee. Aplastic anemia (AA) is defined as hypoplastic marrow with two of the following: neutrophil count <1.5 x 10^9/l, platelet count <100 x 10^9/l, and hemoglobin level <10g/dl. Severe AA (SAA) is defined as hypoplastic marrow with two of the following: neutrophil count <0.5 x 10^9/l, platelet count <20 x 10^9/l, and reticulocyte count <20 x 10^9/l. The onset of BMF was defined as the time when one of the following laboratory parameter values used in the International Fanconi Anemia Registry study[1] was observed: a platelet count <100 X 10^9/L, a hemoglobin level < 10 g/dl, or an absolute neutrophil count <1 X 10^9/L[1].

PCR, sequencing, and Taqman PCR. Genomic DNA or total RNA was isolated from either PHA-stimulated lymphocytes or cultured fibroblasts using Puregene (Qiagen) or RNAeasy (Qiagen) kits, respectively. cDNA was synthesized with a Primescript II cDNA synthesis kit (Life Technologies). Mutation analyses of cDNA and genomic DNA samples regarding *FANCA, FANCC,* or *FANCG* were carried out by PCR and direct sequencing as previously described[2]. In addition, 45 patients were examined by the Multiplex Ligation-mediated Probe Amplification (MLPA) test for *FANCA* (Falco Biosystems). Additional patients (n=29) were screened by whole exome sequencing for mutations in the known 16 FA genes as described below. The *ALDH2* genotype was determined by a previously established Taqman-PCR assay[3].

Whole exome sequencing. For exome sequencing, genomic DNA from each patient was enriched for protein-coding sequences with a SureSelect Human All Exon V4 or V5 kit

(Agilent Technologies, Santa Clara, CA, USA). This was followed by massively parallel sequencing with the HiSeq 2000 platform with 100 bp paired-end reads (Illumina, San Diego, CA, USA). Candidate germline variants were detected through our in-house pipeline for exome-sequencing analysis with minor modifications for the detection of germline variants[4]. The obtained sequences were aligned to the human reference genome (hg19) with the Burrows-Wheeler Aligner (BWA). After removal of duplicate artifacts caused by PCR, the single nucleotide variants with an allele frequency > 0.25 and insertion-deletions with an allele frequency > 0.1 were called. All of the identified variants in the FA genes were verified by PCR and Sanger sequencing.

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Legend for Supplemental Table 1. Clinical characteristics of the patients studied.

MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; SAA, severe aplastic anemia; AA, non-severe aplastic anemia; SCT, hematopoietic stem cell transplantation; NI, not identified; WES, whole exome sequencing; MLPA, Multiplex Ligation-mediated Probe Amplification method.

[A] These mutations underwent reversion to wild type in lymphocytes.
[B] These mutations were presumptive, since their functional significance has not been determined.
[C] FA-A cases in which nonsense mutations, frame-shifts, or large deletions have been identified in both alleles. Progression of BMF in these cases was analyzed and presented in Figure 1C.
[D] This synonymous mutation is predicted to likely disturb normal splicing by Mutation Taster (http://www.mutationtaster.org).
[E] Physical abnormalities included skin abnormalities (hyperpigmentation and *café au lait* spots), low birth weight, growth defects (short stature), and malformations (affecting thumb, radius, skeleton, head/face, eyes, ears, kidneys, gastrointestinal tract, urogenital tract and cardiovascular system). The sum of the number of physical abnormalities and the number of anatomical sites involved in malformations is presented.
[F] Extensive malformation was defined as the involvement of at least three sites including at least one deep organ.
[G] Genomic PCR and sequencing confirmed that the exon27 deletion detected by MLPA was caused by the mutation c.2546delC. This mutation probably abolishes the exon 27 signal because one of the MLPA probes set on exon 27 overlaps with FANCA C2546. In cases #43 and #59, we have not yet determined whether the Exon 27 deletion is associated with a c.2546delC mutation or not.

Supplemental Table 1. Clinical characteristics of the patients studied.

Patient No.	ALDH2 allele	Disease	Defective FA genes	Allele1	Allele2	DNA origin	WES	FANCA-MLPA	Onset of BMF(months)	MDS or AML (months)	SCT (months)	The last observation (months)	Number of physical abnormalities[E]	Extensive malformation[F]	% birth weight
1	AA	MDS	A[C]	c.2546delC (p.S849FfsX40)	c.3781_3785delTTCTT (p.P1261LfsX15)	Blood			7	7	36		3		78.9
2	AA	MDS	A	c.2546delC (p.S849FfsX40)	NI	Blood			0	12	13		5	Yes	79.2
3	AA	MDS	P	c.343delA (p.S115AfsX11)	c.343delA (p.S115AfsX11)	Blood	Yes	Normal	0	4		6	6	Yes	73.8
4	GA	SAA	A	c.2546delC (p.S849FfsX40)	NI	Blood			24		58		5		85.2
5	GA	SAA	A[C]	c.2546delC (p.S849FfsX40)	c.3931-3932delAG (p.S1311X)	Skin-fibroblast		ex27[G]/-	21		69		6	Yes	61.2
6	GA	SAA	A	c.1023G>C (p.Q341H)[B]	c.1639G>T (p.A547S)[B]	Skin-fibroblast		Normal	24		249		3		91.2
7	GA	MDS	A	c.4168-2A>G	c.2546delC (p.S849FfsX40)	Skin-fibroblast		ex27[G]/-	28	168	171		2		69.8
8	GA	SAA/tongue cancer	A[C]	c.2593delA (p.I879LfsX24)	ex18-21 del	Blood		ex18-21/-	53			469	0		71.8
9	GA	SAA	G	c.307+1G>C	c.307+1G>C	Blood			38		56		3		66.5
10	GA	SAA	A[C]	c.2546delC (p.S849FfsX40)	ex3 del	Blood		ex27[G]/ex1-3	22		122		5	Yes	76.4
11	GA	SAA	G	c.307+1G>C	c.307+1G>C	Blood			36		90		2		76.1
12	GA	SAA	A[C]	c.2546delC (p.S849FfsX40)	ex30 del	Blood		ex27[G]/ex30	37		74		1		97.4
13	GA	SAA	A[C]	c.2546delC (p.S849FfsX40)	c.2546delC (p.S849FfsX40)	BM-fibroblast		ex27[G]/ex27[G]	38		80		5		78.9
14	GA	MDS	P	c.2629G>A (p.A877T)[B]	NI	Blood	Yes	Normal	12	108	135		2		98.2
15	GA	MDS	G	c.1066C>T (p.Q356X)	c.307+1G>C	Blood	Yes		12	61	62		5	Yes	82.9
16	GA	SAA	I	c.158-2A>G	c.288G>A (p.E96E)[D]	Blood	Yes	Normal	7		45		11	Yes	52.4
17	GA	SAA	A	c.1303C>T (p.R435C)	c.4168-1G>C	Blood	Yes		26		168		2		53.5
18	GA	MDS	M	c.2330A>G (p.Y777C)[B]	NI	Blood	Yes	Normal	24	51	51		12	Yes	66.2
19	GA	SAA	G	c.307+1G>C	c.307+1G>C	Blood	Yes	Normal	48		88		7	Yes	90.6
20	GA	SAA	G	c.307+1G>C	c.307+1G>C	Blood	Yes		39		84		5		100.6
21	GA	SAA	I	c.3346_3347insT (p.S1116FfsX16)	c.2826+3 A>G[B]	Blood	Yes	Normal	15		28		9	Yes	78.6
22	GA	SAA	A	c.2602-2A>T	c.4198C>T (p.R1400C)	Blood	Yes	Normal	48		154		3		94.9
23	GA	SAA	G	c.907_908delCT (p.L303GfsX5)	c.307+1G>C	Blood	Yes		21		78		1		66.9
24	GA	SAA	G	c.307+1G>C	c.307+1G>C	Blood	Yes		69		135		4	Yes	74

25	GA	SAA	G	c.307+1G>C	c.307+1G>C	Blood	Yes	Normal	18			72	2		85.8
26	GA	MDS	A	ex 30 del	NI	Blood		ex30/-	60	192	199		4	Yes	102.1
27	GG	SAA	A	c.2602-2A>T	c.2527T>G(p.Y843D)	Skin-fibroblast		Normal	78		228		3		83
28	GG	SAA	A[C]	c.2546delC (p.S849FfsX40)	c.2546delC (p.S849FfsX40)	Blood			114		128		3		87.7
29	GG	AML	A	c.2602-2A>T	c.2602-2A>T	Blood		Normal	62	311	316		3		81.5
30	GG	AML	A	c.4124_4125delCA (p.T1375SfsX49)	c.2290C>T (p.R764W)	Blood		Normal	156	156	162		3		90.1
31	GG	SAA	G	c.307+1G>C	c.1066C>T (p.Q356X)	Blood			50		52		4		66.2
32	GG	MDS	A reversion(loss of allele2 mutation)	c.2546delC (p.S849FfsX40)	c.3295C>T[A] (p.Q1099X)	BM-fibroblast		ex27[G]/ex33	49	189	192		6	Yes	72.4
33	GG	MDS/tongue cancer	A reversion(loss of allele1 mutation)	c.44_69delA (p.P15RfsX13)	c.2170A>C (p.T724P)	BM-fibroblast		Normal	108	348		384	2		62.4
34	GG	AML	A	c.2602-1G>A	NI	Blood			108	384	448		3		82.2
35	GG	SAA	A[C]	c.2546delC (p.S849FfsX40)	ex38 del	Blood			72		122		1		92.3
36	GG	SAA	A	ex24-28 skipping	NI	Blood			60		144		4		74.8
37	GG	AML	A	c.1470+?_1626+?del	NI	Blood		ex16-17/-	61	61	66		1		70.6
38	GG	SAA	A	c.2546delC (p.S849FfsX40)	c.4168-2A>G	Blood		ex27[G]/-	106		184		2		70.4
39	GG	SAA	A[C]	c.2546delC (p.S849FfsX40)	c.2546delC (p.S849FfsX40)	Blood		ex27[G]/ex27[G]	70		153		5	Yes	76.6
40	GG	SAA	G	c.307+1G>C	c.307+1G>C	Blood			28		67		0		84.9
41	GG	MDS	A	c.2546delC (p.S849FfsX40)	c.1567-1G>A	Blood		ex27[G]/ex32	82	82	108		3		67.9
42	GG	SAA	A[C]	c.2546delC (p.S849FfsX40)	c.3720_3724del (p.E1240DfsX36)	Blood		ex27[G]/ex37	88		185		2		98.7
43	GG	MDS	A[C]	ex27 del	ex1-28 del	Blood	Yes	ex27/ex1-28	72	72	78		1		84
44	GG	SAA	A	ex30 del	NI	Blood		ex30/-	45		130		4		84
45	GG	SAA	A	ex11-15 duplication	NI	Blood		ex11-15/-	297		318		2		Unknown
46	GG	SAA	A	ex30 del	NI	BM-fibroblast		ex30/-	96		182		2		97
47	GG	MDS	A	c.2870G>A (p.W957X)	c.2723_2725TCT>GCC (p.LS908_909RP	Blood	Yes		121	335	343		3		Unknown
48	GG	MDS	G	c.1386delC (p.A463GfsX55)	c.1637-15 G>A[B]	Blood	Yes	Normal	69	120	133		2		88.1
49	GG	SAA	G	c.1066C>T (p.Q356X)	c.1066C>T (p.Q356X)	Blood	Yes	Normal	66		77		4		70.5
50	GG	SAA	G	c.1066C>T (p.Q356X)	c.1066C>T (p.Q356X)	Blood	Yes	Normal	72		79		1		68.6
51	GG	SAA	G	c.91C>T (p.Q31RfsX5)	c.307+1G>C	Blood	Yes		27		109		4		77

52	GG	SAA	G	c.91C>T (p.Q31RfsX5)	c.307+1G>C	Blood	Yes		60		113		4		66.7
53	GG	AML	A	c.4240_4241delAG (p.S1414LfsX10)	c.2602-1G>A	Blood	Yes	Normal	41	115	128		6	Yes	67
54	GG	SAA	A	c.2602-2A>T	c.4198C>T (p.R1400C)	Blood	Yes	Normal	120		190		3		86.4
55	GG	MDS	A^C (reversion suspected)	c.2546delC (p.S849FfsX40)	c.3919_3920insT (p.Q1307LfsX6)	Blood	Yes	$ex27^G$/-	144	145	208		2		89.4
56	GG	AA	A	c.2602-2A>T	c.2602-1G>A	Blood	Yes	Normal	134			147	2		67.4
57	GG	AA	A	ex30 del	NI	Blood		ex30/-	51			65	3		89.5
58	GG	SAA	A	ex30 del	NI	Blood		ex30/-	92		104		3		81.2
59	GG	AML	A^C	ex27 del	ex37 skipping	Blood		ex27/-	136	176	179		2		Unknown
60	GA	SAA	Not detected	NI	NI	Blood	Yes		87		162		3		67.7
61	GA	AML	Not detected	NI	NI	Blood	Yes	Normal	41	41	99		3		84
62	GG	SAA	Not detected	NI	NI	Blood	Yes	Normal	58		72		10	Yes	57.4
63	GG	SAA	Not detected	NI	NI	Blood	Yes	Normal	40		61		6	Yes	75.7
64	GG	SAA	Not detected	NI	NI	Blood	Yes	Normal	96		106		2		73.1

Legend for Supplemental Table 2. DEB test results of the patients and control subjects.

Achromatic areas less than a chromatid in width were scored as gaps. Exchange configurations, and dicentric and ring chromosomes were scored as rearrangements. Gaps were excluded from the calculation of chromosome aberrations per cell, and rearrangements were scored as 2 breaks.

Supplemental Table 2. DEB test results of the patients and control subjects.

Patient and control number		Spontaneous breakage rate (breaks/cell)	Spontaneous breakage rate (% aberrant cells)	DEB-induced breakage rate (breaks/cell)	DEB-induced breakage rate (% aberrant cells)
Patient	1	0.08	10	6.45	95
	2	0.08	6	0.44	37
	3	0.02	3	0.91	38
	4	0.02	2	1.21	48
	5	0.04	10	1.3	59
	6	0.06	8	8.32	100
	7	0	4	0.36	39
	8	0.04	6	2.28	38
	9	0.02	2	0.79	46
	10	0.02	14	0.82	59
	11	0.09	9	3.16	72
	12	0.02	2	2.66	83
	13	0.02	4	0.64	30
	14	0.05	11.5	0.3	22.9
	15	0.12	16	8.54	100
	16	0	0	0.52	17
	17	0.06	8	0.56	38
	18	0.06	6	4.2	92
	19	0.07	10	3.49	92
	20	0	2	2.36	68
	21	0.04	7	0.96	52.4
	22	0.01	4	4.07	99
	23	0.08	14	6.45	100
	24	0.01	1	6.14	99
	25	0	2	2.68	73

26	0	3	1.17	67
27	0.06	16	0.45	43
28	0.08	12	1.96	60
29	0	0	4.97	84
30	0.02	4	5.16	95
31	0.02	2	4.77	97
32	0	0	0.12	9
33	0	0	0.03	2
34	0.04	4	5.92	97.9
35	0	0	6.15	81
36	0.1	8	9	100
37	0.04	4	7.67	99
38	0.02	6	1.59	72
39	0.05	6	2.06	76
40	0.06	9	1.93	73
41	0	0	1.92	70
42	0.02	2	5.39	97
43	0.04	6	6.38	97
44	0	2	1.72	68
45	0.1	10	6.12	97.3
46	0.02	6	4.92	96
47	0.1	3	4.39	90
48	0.01	5	2.56	82
49	0	6	3.1	91
50	0.04	4	2.54	80
51	0.06	6	0.92	54.4
52	0.04	6	0.93	49.5
53	0.02	3.2	6.3	97.3
54	0.01	2	3.58	92
55	0.01	1	0.01	2
56	0.01	2	1.4	57

	57	0	0	4.85	93
	58	0.01	4	0.59	46
	59	0.02	8	0.67	36.9
	60	0	0	0.48	28
	61	0.01	2	0.89	35
	62	0.49	52	3.8	95.7
	63	0	0	7.8	100
	64	0.09	9	2.38	54
Control	1	0	0	0.02	2
	2	0	0	0.01	1
	3	0	0	0.01	1
	4	0.01	1	0.05	5
	5	0.01	1	0.05	5
	6	0	1	0.01	1
	7	0	2	0.04	4
	8	0	1	0.04	4
	9	0	0	0.02	2
	10	0	0	0.03	3
	11	0	0	0.02	3
	12	0	1	0	2
	13	0	2	0.02	4
	14	0	0	0.02	2
	15	0	1	0	1
	16	0	0	0	1
	17	0	0	0.04	4

18	0	0	0	2
19	0	1	0.05	5
20	0	0	0	0
21	0	2	0.04	6
22	0.02	2	0.04	4
23	0	0	0	1
24	0	0	0.04	5
25	0	0	0	4
26	0	0	0.02	3
27	0.02	2	0	1
28	0	0	0.02	4
29	0	0	0	0
30	0	0	0.03	2
31	0	0	0.07	10
32	0.02	3	0.04	5